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Supplemental Material

Repetitive Ozone Exposures and Evaluation of Pulmonary Inflammation and Remodeling in Diabetic Mouse Strains

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Figure S1. *Body weights after air or ozone exposure for 13 days.* Final body weights of C57BL/6, KK and KKAY mice at the time of necropsy (A), and their change in body weight since the beginning of exposures to the time of necropsy 14 days later (B). C57BL/6J (open box), KK (gray box) and KKAY (black box). Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, p < 0.05. Summary data for panels A, and B can be found in Tables S2, and S3, respectively.

Figure S2. *Serum leptin and adiponectin in fasted mice after air or ozone exposure for 13 days.* Plasma concentrations of leptin (A) and adiponectin (B) were measured from fasted all mice at the time of necropsy, approximately 22 hours after the last O₃ exposure as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAY (black box). Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, p < 0.05. Summary data for panels A, and B can be found in Tables S7, and S8, respectively.

Figure S3. *Histologic assessment of ozone-induced pulmonary infiltration of neutrophils, eosinophils and macrophages in KKAY mice.* The density of neutrophils (A), eosinophils (B), total macrophages (C) and Ym1/2-positive macrophages (D) in lung tissue were immunohistochemically determined in lung tissue by high resolution morphometric methods as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAY (black box). (E) Representative image of lung tissue from KKAY mouse that was stained with major basic protein to identify eosinophils (arrows) in the centriacinar lesions (identified with asterisk). tb = terminal bronchial, ad = alveolar duct, and a = alveoli. Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, p < 0.05. ND = not detected. Summary data for panels A, B, C, and D can be found in Tables S16, S17, S18, S19, respectively.

Figure S4. *α -Smooth Muscle Actin Staining in KKAY mice.* Tissue sections from air-exposed (A) and repetitive O₃-exposed (B) KKAY mice underwent staining for α -smooth muscle actin (SMA) to identify myofibroblasts. α -SMA staining in air-exposed animals is noted in the subendothelial space (dashed arrow), while in the O₃-exposed mice α -SMA staining is noted in the centriacinar regions (solid arrow). Images are representative images. a = alveoli, ad = alveolar duct, e = endothelium.

Figure S5. *Immunofluorescence staining of SFTPC, CCSP and HA in KK and KKAY mice.* Increased sized images of KK and KKAY strains for visualization of the differences in staining and morphology in the air- and O₃-exposed KK and KKAY strains.

Figure S6. *CCSP immunohistology staining and morphometry in C57BL/6J, KK and KKAY mice.* Light photomicrographs of a centriacinar region in the lungs of C57BL/6 mice (A, B), KK mice (C, D) and KKAY mice (E, F) exposed to air (A, C, E) or ozone (B, D, F). Tissues were immunohistochemically stained for Club Cell Secretory Protein (CCSP; solid arrow; red chromagen) in epithelial cells (e) lining the terminal bronchiole (TB). Area of alveolitis (marked with stippled arrow), which included alveolar septal thickening, type two alveolar epithelial hyperplasia and macrophage accumulation in alveolar airspaces observed in the proximal alveolar duct (AD) and adjacent alveolar parenchyma (a). (G) Morphometry quantification of the airway was performed for CCSP in air- and O₃- exposed C57BL/6, KK and KKAY strains. Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, $p < 0.05$. Summary data for panel G can be found in Table S37.

Table S1 - Primers used for real-time PCR.

Gene Symbol	Assay ID	Description (AB gene Name)	NCBI Accession Number:
<i>Actb</i>	Mm00607939_s1	Mouse ACTB (actin, beta) Endogenous Control (FAM™ Dye/MGB Probe, Non-Primer Limited)	NM_007393
<i>Gapd</i>	Mm99999915_g1	Mouse GAPD (GAPDH) Endogenous Control (FAM/MGB Probe, Non- Primer Limited)	NM_008084
<i>Gusb</i>	Mm00446953_m1	glucuronidase, beta	NM_010368
<i>Arg1</i>	Mm00475988_m1	arginase type I	NM_007482
<i>Ccl8</i>	Mm01297183_m1	chemokine (C-C motif) ligand 8	NM_021443
<i>Ccl11</i>	Mm00441238_m1	chemokine (C-C motif) ligand 11	NM_011330
<i>Chia1</i>	Mm00458221_m1	chitinase, acidic	NM_023186
<i>IL-13</i>	Mm00434204_m1	interleukin 13	NM_008355
<i>Mmp12</i>	Mm00500554_m1	matrix metalloproteinase 12	NM_008605
<i>Saa3</i>	Mm00441203_m1	serum amyloid A3	NM_011315
<i>Scgb1a1</i>	Mm00442046_m1	secretoglobin, family 1A, member 1 (uteroglobin)	NM_011681

Notes: List of all primers for gene expression analysis using the TaqMan gene expression assay from Applied Biosystems.

Tabular Data of Figures:

All data are expressed as Mean \pm SEM. Statistical differences are indicated where $p \leq 0.05$. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air. For gene expression analysis, *= significantly different from respective strain exposed to filtered air.

Table S2 – Data for Figure S1A: Body Weight (g)

Group	Mean	SEM	n/group
Air/C57BL/6J	20.0	0.42	8
Air/KK	26.88 ^a	0.97	8
Air/KKAy	27.98 ^a	0.25	8
O ₃ /C57BL/6J	19.88	0.88	8
O ₃ /KK	26.27 ^a	0.62	8
O ₃ /KKAy	28.64 ^{a,b}	0.35	8

Table S3 – Data for Figure S1B: Change in Body Weight (g)

Group	Mean	SEM	n/group
Air/C57BL/6J	5.0	0.87	8
Air/KK	11.25 ^a	0.84	8
Air/KKAy	13.57 ^{a,b}	0.28	8
O ₃ /C57BL/6J	5.0	0.96	8
O ₃ /KK	8.375 ^{a,c}	0.62	8
O ₃ /KKAy	10.63 ^{a,b,c}	0.71	8

Table S4 – Data for Figure 1A: Blood Glucose (mg/dL)

Group	Mean	SEM	n/group
Air/C57BL/6J	148.8	11.8	8
Air/KK	347.3 ^a	8.9	7
Air/KKAy	393.4 ^a	39.9	8
O ₃ /C57BL/6J	157.8	6.6	8
O ₃ /KK	355.9 ^a	37.0	8
O ₃ /KKAy	403.1 ^a	23.3	8

Table S5 – Data for Figure 1B: Plasma Insulin (μ U/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	32.9	11.8	8
Air/KK	121.2 ^a	8.9	7
Air/KKAy	240.8 ^{a,b}	39.9	8
O ₃ /C57BL/6J	17.5 ^c	6.6	7
O ₃ /KK	66.3 ^{a,c}	37.0	7
O ₃ /KKAy	153.5 ^{a,b,c}	23.3	8

Table S6 – Data for Figure 1C: HOMA-IR

Group	Mean	SEM	n/group
Air/C57BL/6J	11.7	2.1	8
Air/KK	103.7 ^a	11.8	7
Air/KKAy	234.1 ^{a,b}	40.0	8
O ₃ /C57BL/6J	6.8	0.9	7
O ₃ /KK	57.1 ^{a,c}	7.8	7
O ₃ /KKAy	147.0 ^{a,b}	21.3	8

Table S7 – Data for Figure S2A: Plasma Leptin (ng/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	1.25	0.12	8
Air/KK	14.84 ^a	1.78	8
Air/KKAy	25.39 ^{a,b}	1.25	8
O ₃ /C57BL/6J	1.40	0.17	8
O ₃ /KK	8.27 ^{a,c}	0.70	8
O ₃ /KKAy	21.61 ^{a,b}	1.35	8

Table S8 – Data for Figure S2B: Plasma Adiponectin (ng/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	6204.38	483.67	8
Air/KK	2862.17 ^a	108.45	8
Air/KKAy	2822.32 ^a	124.00	8
O ₃ /C57BL/6J	7459.41	979.10	8
O ₃ /KK	3327.95 ^a	101.93	8
O ₃ /KKAy	3049.00 ^a	210.96	8

Table S9 – Data for Figure 1D: Blood Glucose (mg/dL)

Group	t=0min		15min		30min		60min		90		120		130		n/group
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Air/C57BL6J	148.8	11.8	122.1	19.6	96.3	15.0	96.3	16.5	110.9	13.6	114.1	11.1	126.3	14.1	8
Air/ KK	347.3	8.9	255.1	14.6	218.9	19.9	195.5	23.6	210.1	17.2	199.4	17.9	210.6	18.0	8
Air/KKAy	393.4	39.9	328.5	27.3	286.5	24.0	280.4	26.7	275.6	24.9	262.3	12.6	272.5	17.0	8
O ₃ /C57BL6J	158.6	5.7	107.3	12.8	89.3	13.8	58.1	6.0	74.3	8.8	85.8	6.1	90.5	5.0	8
O ₃ /KK	356.6	32.1	360.2	26.7	354.4	26.7	335.4	33.6	331.6	41.3	272.3	30.2	259.5	29.7	8
O ₃ /KKAy	403.1	23.3	400.1	15.2	344.0	14.2	349.6	32.3	323.3	33.1	293.8	28.8	268.1	17.1	8

Table S10 – Data for Figure 1E: Blood Glucose (AUC)

Group	Mean	SEM	n/group
Air/C57BL/6J	14,240	596.5	8
Air/KK	28,566 ^a	782.2	8
Air/KKAy	37,613 ^{a,b}	991	8
O ₃ /C57BL/6J	10,949	367.1	8
O ₃ /KK	42,805 ^{a,c}	1365	8
O ₃ /KKAy	44,168 ^a	1136	8

Table S11 – Data for Figure 2A: Total Cells (cells /ml x 10⁵)

Group	Mean	SEM	n/group
Air/C57BL/6J	82,500	7,102	8
Air/KK	125,938 ^a	17,307	8
Air/KKAy	132,500 ^a	12,344	7
O ₃ /C57BL/6J	214,688 ^c	31,695	8
O ₃ /KK	362,500 ^{a,c}	40,551	8
O ₃ /KKAy	521,250 ^{a,b,c}	58,542	8

Table S12 – Data for Figure 2B: Macrophages (cells /ml x 10⁵)

Group	Mean	SEM	n/group
Air/C57BL/6J	81,978	7,052	8
Air/KK	116,403	16,276	8
Air/KKAy	127,287	13,745	7
O ₃ /C57BL/6J	205,763 ^c	29,062	8
O ₃ /KK	295,078 ^{a,c}	29,793	8
O ₃ /KKAy	339,597 ^{a,c}	25,447	8

Table S13 – Data for Figure 2C: Eosinophils (cells /ml x 10⁴)

Group	Mean	SEM	n/group
Air/C57BL/6J	0	0	8
Air/KK	0	0	7
Air/KKAy	0	0	7
O ₃ /C57BL/6J	646 ^c	244	7
O ₃ /KK	27,451 ^{a,c}	9,126	8
O ₃ /KKAy	124,416 ^{a,b,c}	33,246	8

Table S14 – Data for Figure 2D: Neutrophils (cells /ml x 10⁴)

Group	Mean	SEM	n/group
Air/C57BL/6J	73	126	7
Air/KK	3,703	2,648	7
Air/KKAy	5,440	4,188	7
O ₃ /C57BL/6J	5,657 ^c	5,941	8
O ₃ /KK	30,638 ^{a,c}	13458	8
O ₃ /KKAy	33,318 ^{a,c}	15123	8

Table S15 – Data for Figure 2E: Lymphocytes (cells /ml x 10⁴)

Group	Mean	SEM	n/group
Air/C57BL/6J	422	48	7
Air/KK	2,285	568	7
Air/KKAy	272	122	7
O ₃ /C57BL/6J	1,119	333	7
O ₃ /KK	9,282 ^{a,c}	1,366	8
O ₃ /KKAy	23,849 ^{a,b,c}	4,127	8

Table S16 – Data for Figure S3A: Neutrophils in Lung Tissue (%)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.08	0.04	8
Air/KK	0.37 ^a	0.12	8
Air/KKAy	0.70 ^{a,b}	0.16	8
O ₃ /C57BL/6J	0.03	0.02	8
O ₃ /KK	0.50 ^a	0.056	8
O ₃ /KKAy	1.12 ^{a,b,c}	0.28	8

Table S17 – Data for Figure S3B: Eosinophils in Lung Tissue (%)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.03	0.02	8
Air/KK	0.00	0.00	8
Air/KKAy	0.09	0.04	8
O ₃ /C57BL/6J	0.04	0.04	8
O ₃ /KK	0.62 ^{a,c}	0.16	8
O ₃ /KKAy	3.27 ^{a,b,c}	0.84	8

Table S18 – Data for Figure S3C: Macrophages in Lung Tissue (%)

Group	Mean	SEM	n/group
Air/C57BL/6J	8.05	1.03	8
Air/KK	4.78 ^a	0.34	8
Air/KKAy	6.40	0.78	8
O ₃ /C57BL/6J	5.41 ^c	0.53	8
O ₃ /KK	7.18 ^c	0.80	8
O ₃ /KKAy	6.80	0.47	8

Table S19 – Data for Figure S3D: YM 1/2 Macrophages (% total macrophages)

Group	Mean	SEM	n/group
Air/C57BL/6J	36.46	0.84	8
Air/KK	50.77	7.01	8
Air/KKAy	55.55 ^a	5.69	8
O ₃ /C57BL/6J	55.16 ^c	3.70	8
O ₃ /KK	77.12 ^{a,c}	0.80	8
O ₃ /KKAy	57.63 ^b	6.72	8

Table S20 – Data for Figure 3A: BALF IL-5 (pg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.16	0.21	8
Air/KK	0.00	0.00	8
Air/KKAy	0.00	0.00	8
O ₃ /C57BL/6J	0.58 ^c	0.15	8
O ₃ /KK	1.69 ^c	0.48	8
O ₃ /KKAy	7.05 ^{a,b,c}	1.95	8

Table S21 – Data for Figure 3B: BALF IL-13 (fg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.00	0.00	8
Air/KK	27.66 ^a	8.31	8
Air/KKAy	50.55 ^{a,b}	8.03	8
O ₃ /C57BL/6J	0.00	0.00	8
O ₃ /KK	27.20 ^a	6.85	8
O ₃ /KKAy	84.72 ^{a,b,c}	11.33	8

Table S22 – Data for Figure 3C: BALF IL-6 (pg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.00	0.00	8
Air/KK	0.00	0.00	8
Air/KKAy	0.00	0.00	8
O ₃ /C57BL/6J	0.00	0.00	8
O ₃ /KK	755.92 ^{a,c}	182.08	8
O ₃ /KKAy	25636.05 ^{a,b,c}	12410.26	8

Table S23 – Data for Figure 3D: BALF KC (pg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	0.00	0.00	7
Air/KK	8.96 ^a	1.41	7
Air/KKAy	8.15 ^{a,b}	0.78	7
O ₃ /C57BL/6J	0.00	0.00	7
O ₃ /KK	5.94 ^a	0.62	8
O ₃ /KKAy	4.68 ^{a,c}	0.61	8

Table S24 – Data for Figure 3E: BALF IL-17 (fg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	136.78	9.00	8
Air/KK	31.37 ^a	8.23	8
Air/KKAy	29.81 ^a	9.19	8
O ₃ /C57BL/6J	106.76	10.27	8
O ₃ /KK	36.30 ^a	11.50	8
O ₃ /KKAy	115.31 ^{b,c}	13.47	8

Table S25 – Data for Figure 3F: BALF IL-1 β (fg/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	70.26	17.09	8
Air/KK	44.06	14.65	7
Air/KKAy	51.70	19.90	8
O ₃ /C57BL/6J	117.83	24.45	8
O ₃ /KK	25.74 ^a	7.96	8
O ₃ /KKAy	84.02 ^b	20.81	8

Table S26 – Data for Figure 5G: Tissue Collagen (% lung parenchyma)

Group	Mean	SEM	n/group
Air/C57BL/6J	2.41	0.50	8
Air/KK	3.71 ^a	0.36	8
Air/KKAy	3.94 ^a	0.35	8
O ₃ /C57BL/6J	3.02	0.78	8
O ₃ /KK	6.30 ^{a,c}	0.78	8
O ₃ /KKAy	12.48 ^{a,b,c}	2.05	8

Table S27 – Data for Figure 5I: Lung Hydroxyproline (μ g/ml)

Group	Mean	SEM	n/group
Air/C57BL/6J	52.54	3.92	8
O ₃ /C57BL/6J	61.66	5.74	8

Table S28 – Data for Figure 5H: Lung Hydroxyproline ($\mu\text{g/ml}$)

Group	Mean	SEM	n/group
Air/KK	25.9	2.16	8
Air/KKAy	27.59	2.44	8
O ₃ /KK	24.52	1.105	8
O ₃ /KKAy	38.66 ^c	2.153	8

Table S29 – Data for Figure 6A: Eotaxin mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.09	8
Air/KK	1.00	0.11	8
Air/KKAy	1.00	0.08	8
O ₃ /C57BL/6J	1.23	0.10	8
O ₃ /KK	1.29	0.13	8
O ₃ /KKAy	1.97*	0.24	8

Table S30 – Data for Figure 6B: IL-13 mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.30	8
Air/KK	1.00	0.32	8
Air/KKAy	1.00	0.37	8
O ₃ /C57BL/6J	1.34	0.32	8
O ₃ /KK	1.54	0.32	8
O ₃ /KKAy	6.91*	1.63	8

Table S31 – Data for Figure 6C: *Mcp2* mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.13	8
Air/KK	1.00	0.30	8
Air/KKAy	1.00	0.18	8
O ₃ /C57BL/6J	2.00	0.60	8
O ₃ /KK	1.59	0.30	8
O ₃ /KKAy	5.50*	1.07	8

Table S32 – Data for Figure 6D: *Arg1* mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.09	8
Air/KK	1.00	0.04	8
Air/KKAy	1.00	0.10	8
O ₃ /C57BL/6J	0.70*	0.09	8
O ₃ /KK	1.72*	0.16	8
O ₃ /KKAy	4.07*	1.34	8

Table S33 – Data for Figure 6E: *Mmp12* mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.09	8
Air/KK	1.00	0.08	8
Air/KKAy	1.00	0.10	8
O ₃ /C57BL/6J	1.06	0.22	8
O ₃ /KK	2.55*	0.32	8
O ₃ /KKAy	2.98*	0.40	8

Table S34 – Data for Figure 6F: CCSP mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.06	8
Air/KK	1.00	0.05	8
Air/KKAy	1.00	0.03	8
O ₃ /C57BL/6J	-1.14	0.14	8
O ₃ /KK	-1.12	0.24	8
O ₃ /KKAy	-1.95*	0.22	8

Table S35 – Data for Figure 6G: Chia1 mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.06	8
Air/KK	1.00	0.03	8
Air/KKAy	1.00	0.10	8
O ₃ /C57BL/6J	1.21	0.18	8
O ₃ /KK	0.96	0.06	8
O ₃ /KKAy	2.74*	0.38	8

Table S36 – Data for Figure 6H: Saa3 mRNA

Group	Mean	SEM	n/group
Air/C57BL/6J	1.00	0.57	8
Air/KK	1.00	0.16	8
Air/KKAy	1.00	0.08	8
O ₃ /C57BL/6J	9.00*	2.22	8
O ₃ /KK	6.80*	0.72	8
O ₃ /KKAy	3.57*	0.27	8

Table S37 – *Data for Figure S5G CCSP in Airway Epithelium (%)*

Group	Mean	SEM	n/group
Air/C57BL/6J	81.0	2.5	8
Air/KK	54.6 ^a	1.7	7
Air/KKAy	31.6 ^{a,b}	4.1	8
O ₃ /C57BL/6J	157.8	6.6	8
O ₃ /KK	49.1 ^a	2.0	8
O ₃ /KKAy	31.0 ^{a,b}	2.5	8

Supplemental Figures:

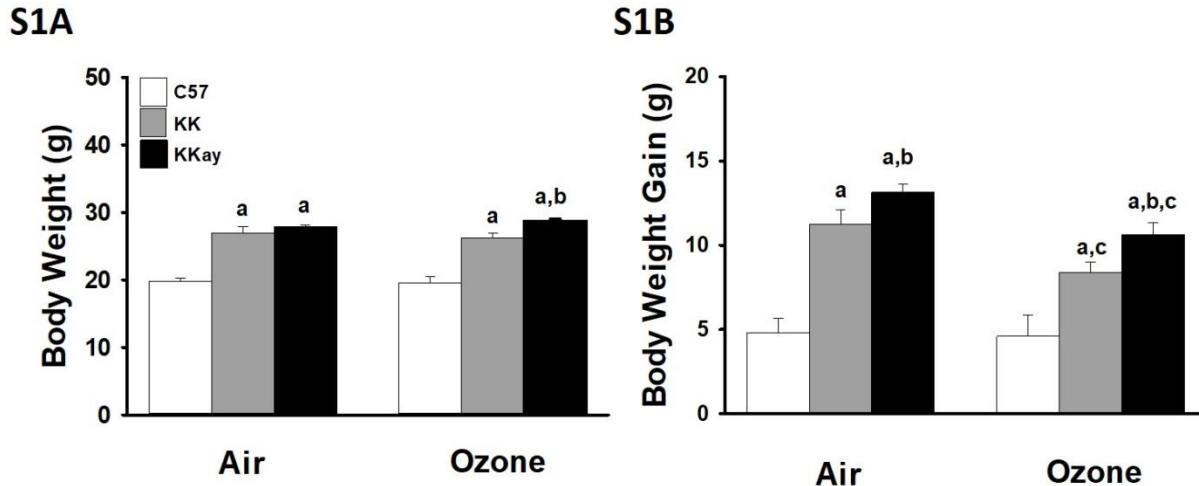


Figure S1 - Body weights after air or ozone exposure for 13 days. Final body weights of C57BL/6, KK and KKAY mice at the time of necropsy (A), and their change in body weight since the beginning of exposures to the time of necropsy 14 days later (B). C57BL/6J (open box), KK (gray box) and KKAY (black box). Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, $p < 0.05$. Summary data for panels A, and B can be found in Tables S2, and S3, respectively

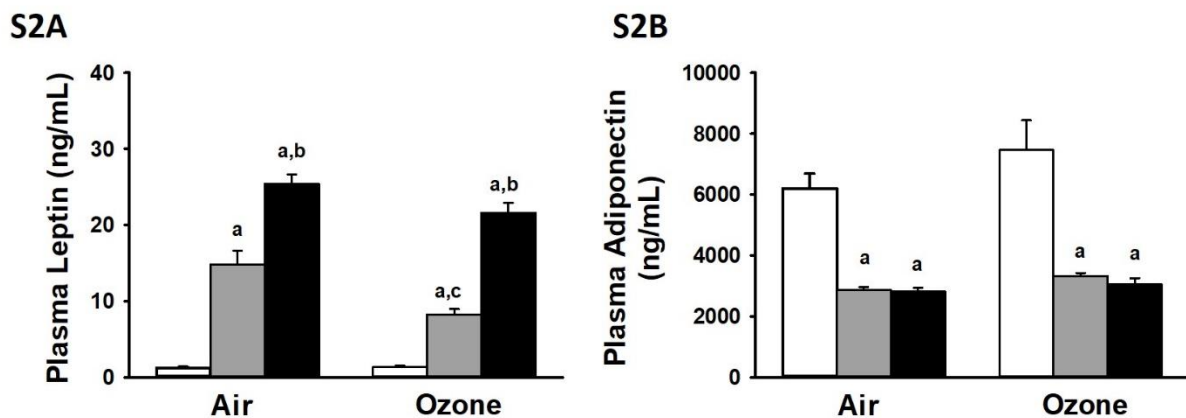
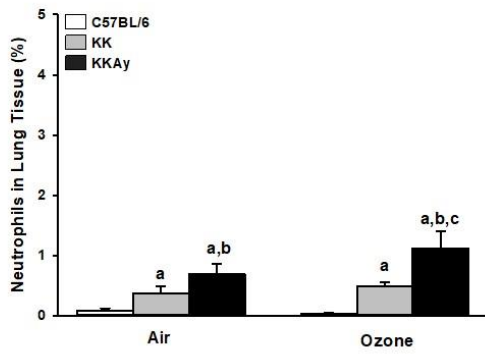
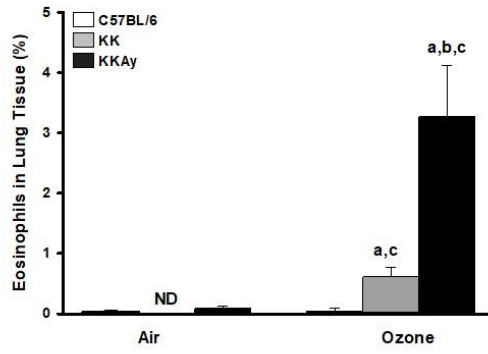


Figure S2 - Serum leptin and adiponectin in fasted mice after air or ozone exposure for 13 days. Plasma concentrations of leptin (A) and adiponectin (B) were measured from fasted all mice at the time of necropsy, approximately 22 hours after the last O₃ exposure as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAy (black box). Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, p < 0.05. Summary data for panels A, and B can be found in Tables S7, and S8, respectively

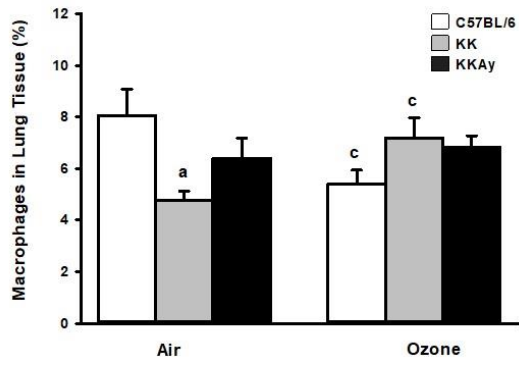
S3A



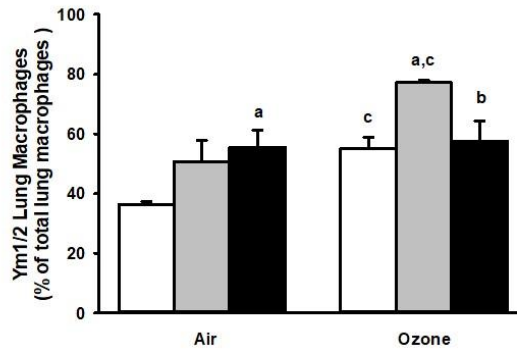
S3B



S3C



S3D



S3E

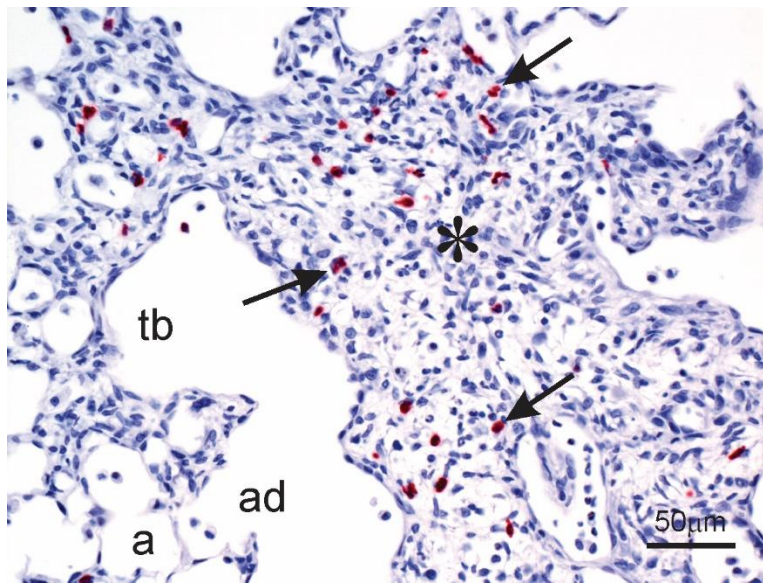


Figure S3 – *Histologic assessment of ozone-induced pulmonary infiltration of neutrophils, eosinophils and macrophages in KKAY mice.* The density of neutrophils (A), eosinophils (B), total macrophages (C) and Ym1/2-positive macrophages (D) in lung tissue were immunohistochemically determined in lung tissue by high resolution morphometric methods as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAY (black box). (E) Representative image of lung tissue from KKAY mouse that was stained with major basic protein to identify eosinophils (arrows) in the centriacinar lesions (identified with asterisk). tb = terminal bronchial, ad = alveolar duct, and a = alveoli. Data are expressed as mean \pm SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, p < 0.05. ND = not detected. Summary data for panels A, B, C, and D can be found in Tables S16, S17, S18, S19, respectively

S4A-B

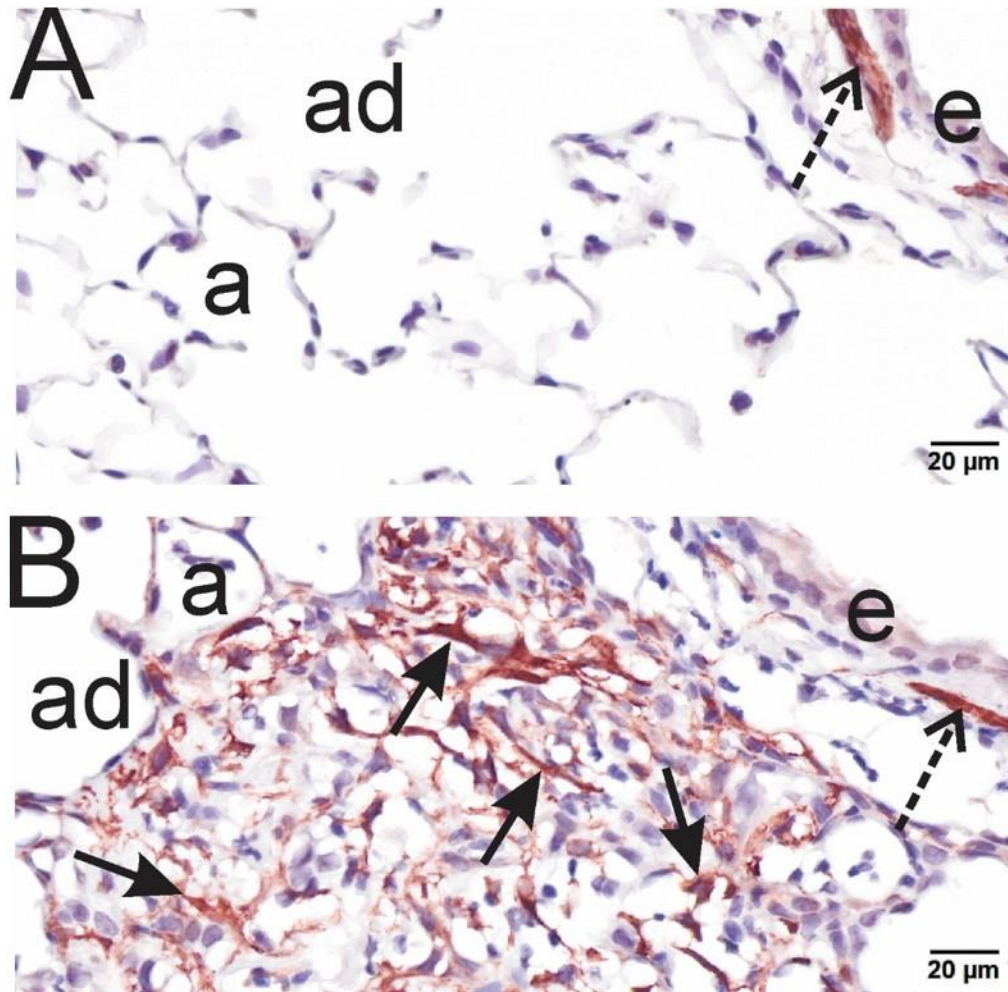


Figure S4 – *α-Smooth Muscle Actin Staining in KKAY mice*. Tissue sections from air-exposed (A) and repetitive O₃-exposed (B) KKAY mice underwent staining for α -smooth muscle actin (SMA) to identify myofibroblasts. α -SMA staining in air-exposed animals is noted in the subendothelial space (dashed arrow), while in the O₃-exposed mice α -SMA staining is noted in the centriacinar regions (solid arrow). Images are representative images. a = alveoli, ad = alveolar duct, e = endothelium.

S5

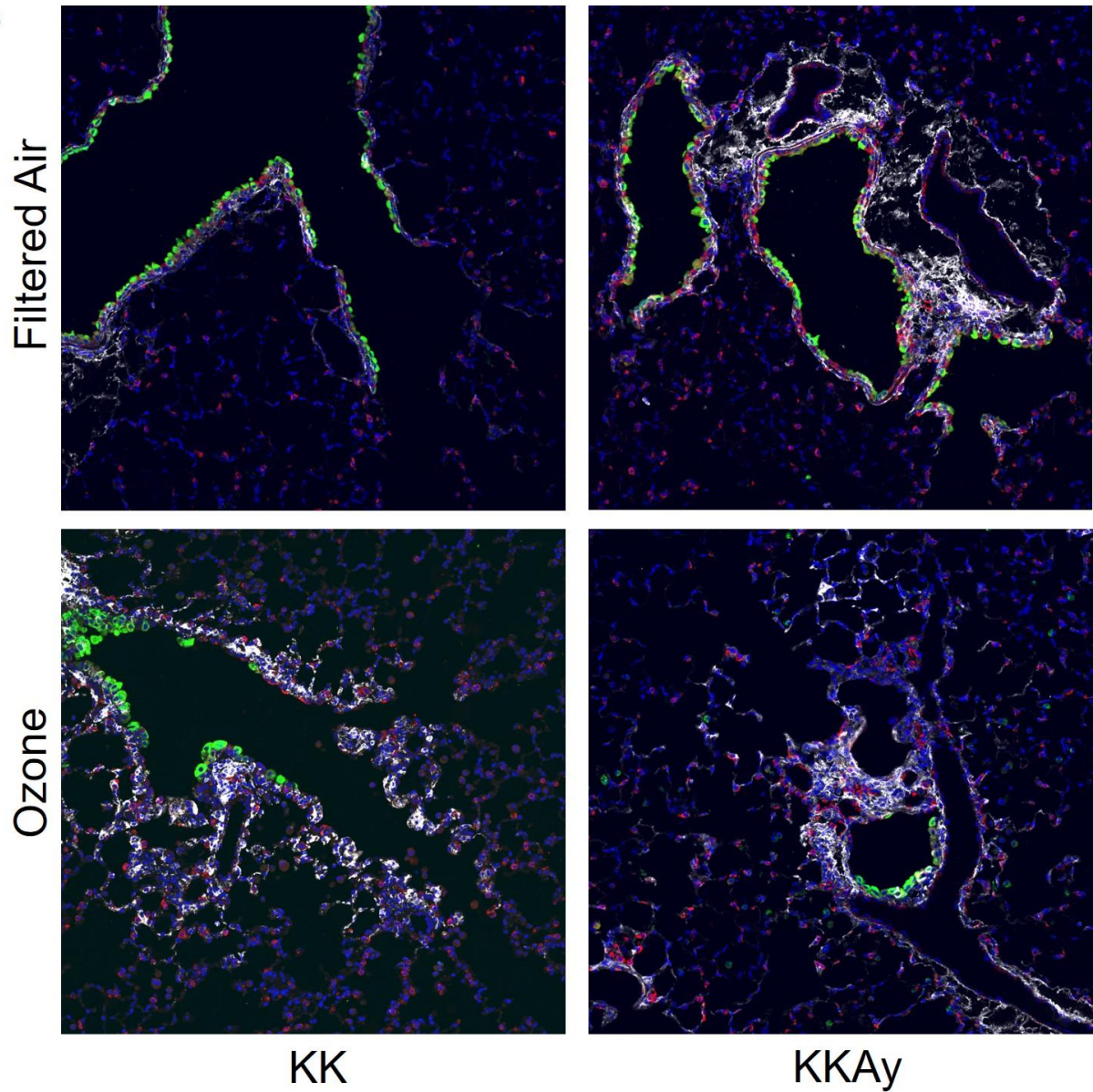
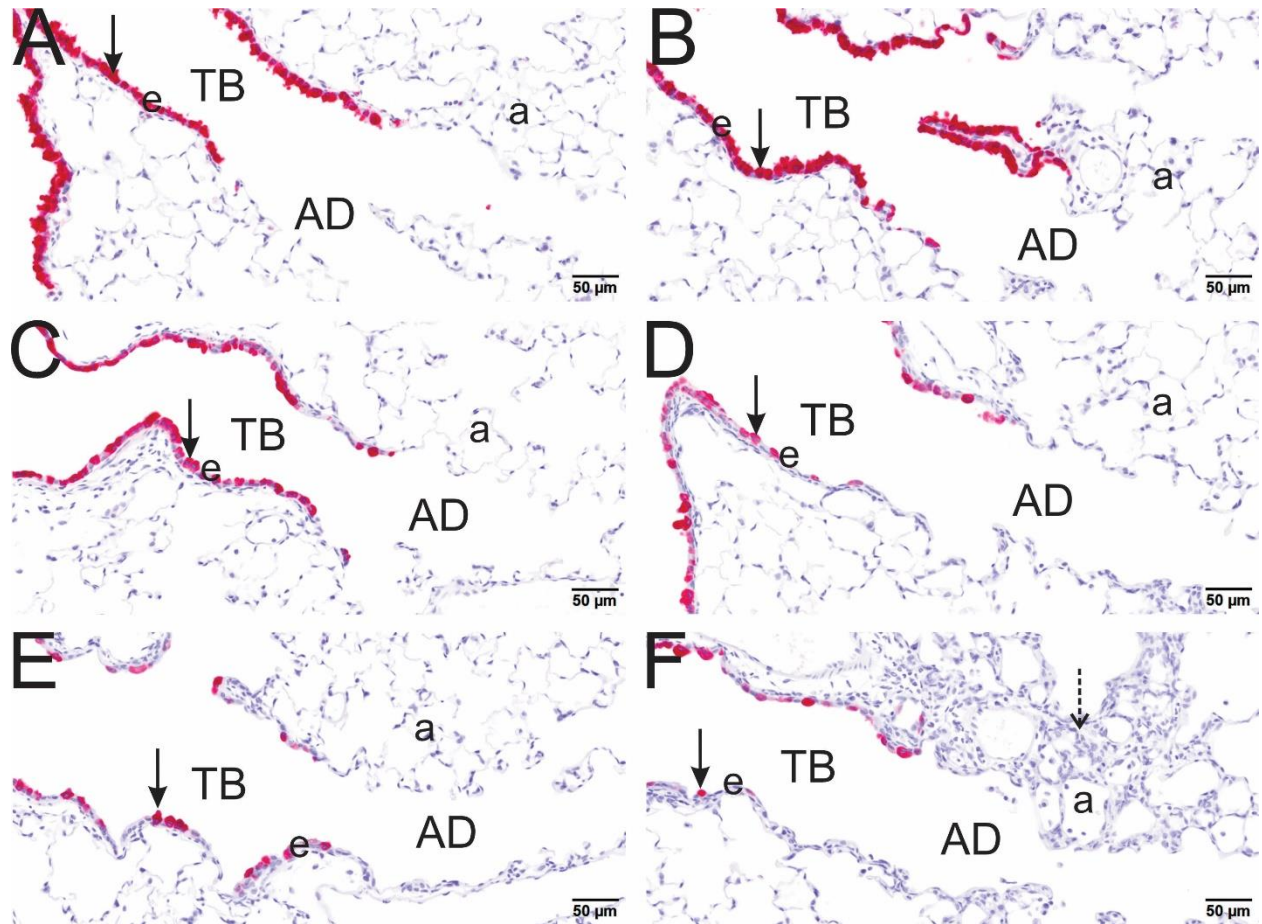


Figure S5 – Immunofluorescence staining of SFTPC, CCSP and HA in KK and KKAY mice. Increased sized images of KK and KKAY strains for visualization of the differences in staining and morphology in the air- and O₃-exposed KK and KKAY strains.



S6G

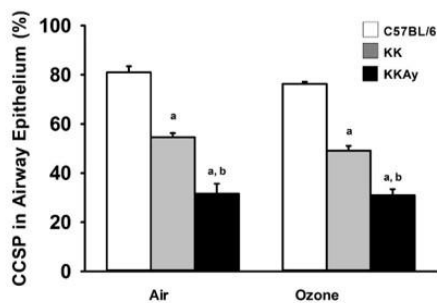


Figure S6 – CCSP immunohistology staining and morphometry in C57BL/6J, KK and KKAY mice. Light photomicrographs of a centriacinar region in the lungs of C57BL/6 mice (A, B), KK mice (C, D) and KKAY mice (E, F) exposed to air (A, C, E) or ozone (B, D, F). Tissues were immunohistochemically stained for Club Cell Secretory Protein (CCSP; solid arrow; red chromagen) in epithelial cells (e) lining the terminal bronchiole (TB). Area of alveolitis (marked with stippled arrow), which included alveolar septal thickening, type two alveolar epithelial hyperplasia and macrophage accumulation in alveolar airspaces observed in the proximal alveolar duct (AD) and adjacent alveolar

parenchyma (a). (G) Morphometry quantification of the airway was performed for CCSP in air- and O₃- exposed C57BL/6, KK and KKAY strains. Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, p < 0.05. Summary data for panel G can be found in Table S37